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Conformational heterogeneity of the aspartate transporter Glt_{Ph}

Supplementary data

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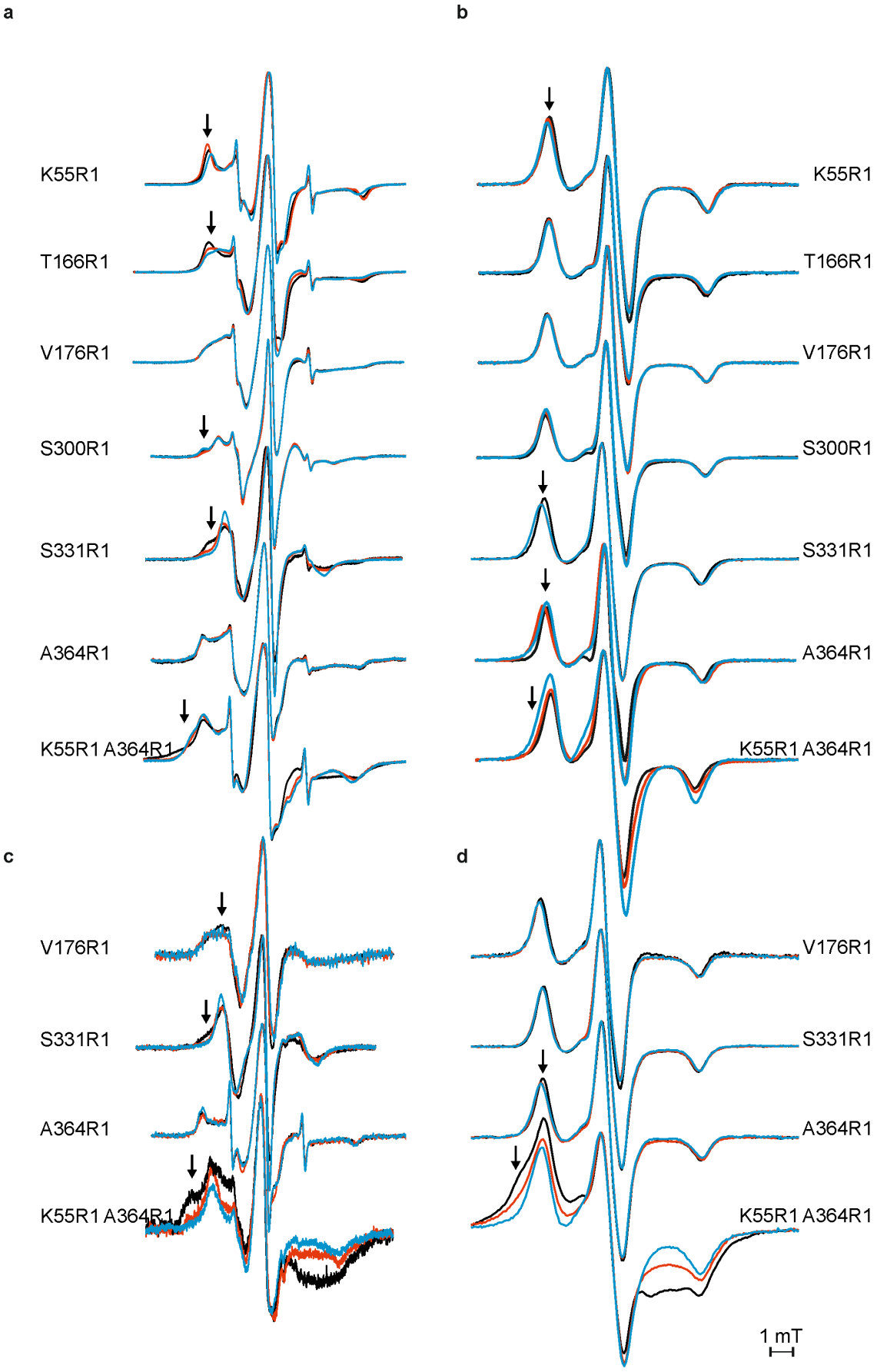

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Supplementary Fig. 1 Continuous wave EPR spectra measured at X band (9.4 GHz). **(a-d)** Spectra of the apoprotein (black lines), in the presence of Na^+ (red lines) and in the presence of Na^+ and aspartate (blue lines). **(a,b)** detergent solubilized spin-labeled proteins; **(c,d)** membrane reconstituted proteins. **(a,c)** Spectra recorded at room temperature; **(b,d)** Spectra recorded at 160 K. The arrows highlight spectral differences comparing the three conditions.

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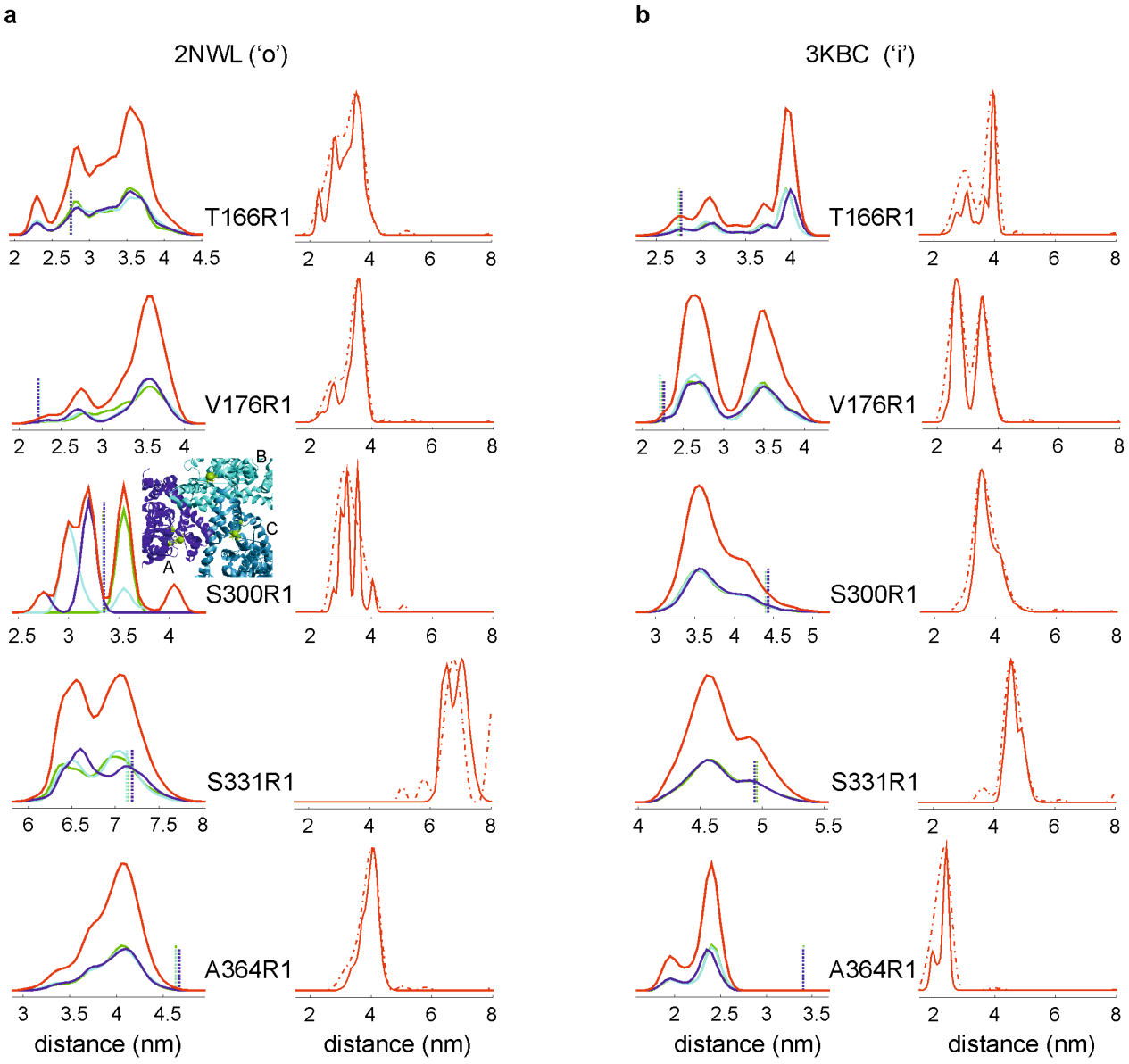

Supplementary Fig. 2 Inter-protomer distances and three-spin effects revealed by rotamer library analysis. (**a**) Left column: inter-protomer distance distributions P(d) obtained by the rotamer library analysis on the outward-facing conformation (PDB 2NWL) for the A-B (green), B-C (blue) and C-A (cyan) protomers. In red the sum of the contributions of the three protomer pairs (as in Fig. 2 in the main text). The vertical lines represent the C_α-C_α distance between the spin-labeled residues. For S300R1 only two rotamers are populated in protomers A and C, and one in protomer B (green rotamers highlighted in the inset), thus each pair of subunits revealed a different interspin distance. Right column: three-spin artifacts on the distance distribution calculated for 100% labeling efficiency (red dash-dotted, Tikhonov regularization parameter 100) compared to the "true" inter-protomer distances (red lines). (**b**) Same analysis performed

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on the inward-facing conformation (PDB 3KBC). Because the artifacts only marginally affected the distributions in all positions investigated and the experimental spin labeling efficiency was less than 100% for most mutants, we did not further consider the three-spin effects.

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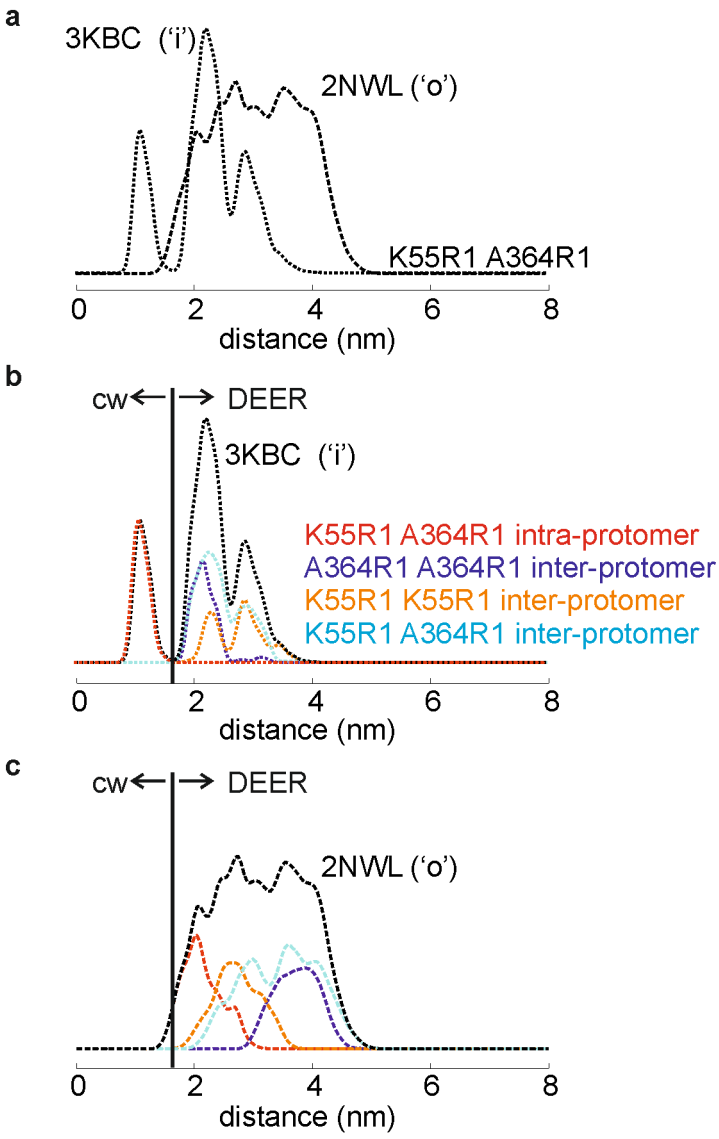

Supplementary Fig. 3 Inter- and intra-protomer distances for the doubly-labeled mutant K55R1 A364R1 revealed by rotamer library analysis. (**a**) Interspin distance distributions obtained by the rotamer library analysis on the outward- (black dashed line) and inward- (black dotted line) facing conformations (PDB 2NWL, 3KBC, respectively). (**b, c**) The same distance distribution as in panel (a) (black) is shown together with the different components arising from intra- and inter-protomer distances, color-coded as indicated in the inset. The vertical line represents the sensitivity threshold between CW and pulsed EPR techniques for reliable distance determination.

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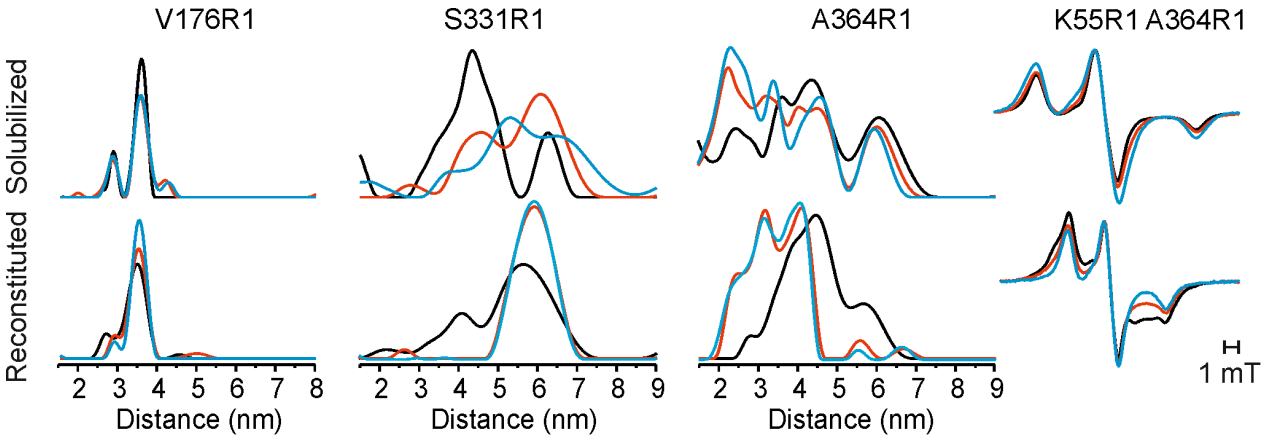

Supplementary Fig. 4 Interspin distance measurements in detergent micelles compared to liposomes. Comparison of interspin distances in detergent-solubilized (data from Figures 2 and 3) and liposome-reconstituted forms (from Figure 4). Apoprotein (black lines), in the presence of Na^+ (red lines), or Na^+ and aspartate (blue lines). For the doubly-labeled mutant K55R1 A364R1 the intensity-normalized low temperature continuous wave EPR spectra are shown.

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Residue	A_{zz} (mT)			Labeling efficiency (%)
	-	+ NaCl	+ NaCl and Aspartate	
K55R1	3.60	3.65	3.68	100
T166R1	3.61	3.61	3.61	100
V176R1	3.61	3.61	3.61	100
S300R1	3.66	3.66	3.63	80
S331R1	3.69	3.69	3.69	61
A364R1	3.62	3.55	3.56	75
K55R1 A364R1	3.51	3.56	3.59	N.D.

Supplementary Table 1. Hyperfine splitting A_{zz} values and labeling efficiency. A_{zz} values are given in mT as determined from low temperature cw EPR measurements shown in Supplementary Fig. 1 with DipFit. A_{zz} reflects the polarity of the environment of the spin label with 3.3 mT corresponding to a very apolar, and 3.7 mT to a very polar surrounding. The error in the A_{zz} is estimated to be ± 0.01 mT. Labeling efficiencies were calculated for singly-labeled variants by double integration of the cw EPR spectra. N.D., not determined.

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